No fee is believed to be due for this submission. Should any fees be due, however, please charge such fees to Winston & Strawn Deposit Account No. 501-814.

Respectfully submitted,

Date: July 31, 2001

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EXHIBIT A
MARKED VERSION OF REPLACEMENT PARAGRAPH

IN THE SPECIFICATION

A marked up version of the following amended paragraphs is attached hereto as Exhibit A. Underling indicates matter that has been added to the paragraph.

Please amend the specification as follows:

On page 8, starting at line 3, please replace the paragraph starting with "**Figure 1**" with the following new paragraph:

Figure 1 is an illustration of a composition of single-domain VH library: nucleotide (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2).

On page 18, starting at line 23, please replace the paragraph starting with "The gene encoding" with the following new paragraph:

The gene encoding the VH domain scaffold [family I(A)] originated from a mouse hybridoma specific for H-2D^{d} + RGPGRAFVTI peptide (SEQ ID NO:3). The 5' region of the VH gene was amplified by PCR using oligonucleotides *SfiI5'* (SEQ ID NO:4) [5'-AAGGAAAAAAGGCCCAGCCGGCCGATGTCCAGCTGCAGGAGTCAGGACCGGC-3'] which introduced the *SfiI* cloning site and the 3' region with *NotI3'* oligonucleotide (SEQ ID NO:5) [5'-TATCAAATGCGGCCGCGACGGTGACAGTGGTCCCTTGGCCCCAGTAGTCMNNMNNMNNNMNNMNNMNNMNNMNNMNNTCTTGCACAGTAATATGTGGCTGT-3'] that randomized 9 amino acids in CDR3 and introduced a *NotI* cloning site.

On page 19, starting at line 1, please replace the paragraph starting with "One μg of PCR" with the following new paragraph:

One μg of PCR product was re-amplified (10 cycles) with the following oligonucleotides *SfiI5'* short (bases 1-26 of SEQ ID NO:4) [5'-AAGGAAAAAAGGCCCAGCCGGCCGATGTCC-3'] and *NotI3'* short (bases1-30 of SEQ ID

NO:5) [5'-TATCAAATGCGGCCGCGACGGTGACAGTGG-3'] to avoid non-symmetric pairing of strands due to primer exhaustion. The final PCR product was digested with *SfiI* and *NotI* and ligated into the phagemid vector pCANTAB 5 E (Amersham Pharmacia Biotech). Ligated DNA was electroporated into the *E. coli* strain TG1 (Gibco BRL).

On page 20, starting at line 24, to and including line 15 on page 21, please replace the paragraph starting with "The VH library" with the following new paragraph:

The VH library was also used in a panning experiment in which biotinylated IgG was immobilized on Streptavidine-coated magnetic beads and Ig binding phage clones where isolated. As shown in Table 1B, after four rounds of panning a 150-fold enrichment in the number of phage captured by antigen was observed. Phage ELISA of individual clones revealed strong and specific binding of the antigen compared to control phage (Figure 3B). The genes encoding the VH protein were rescued from positive phage clones and their sequences were analyzed. As shown in Table 2, all clones exhibited an intact VH insert that contained a random 9 amino acid stretch at the expected location of CDR3. Sequence analysis revealed also consensus residues between positive clones that were isolated after the fourth round of panning. For example, clones number 1 and 4 which recognize Ig show a consensus sequence of GLY-X-SER-PRO-GLN (SEQ ID NO:6). It may be noted that in these cases X is a hydrophilic residue though this may not be a straight requirement. The difference is the location of the consensus within the CDR. For a detailed characterization of a VH single domain protein we choose to use clone 4. Consensus sequences were also obtained in several independent screenings in which antigens were immobilized on polystyrene latex beads and binding phage clones were characterized and found to be specific for plastic (polystyrene). This phenomena is characterized in the literature using peptide phage display libraries and consensus sequences rich in Trp and Tyr, which bind plastic (Adey et al. *ibid*). Several VH phage clones with such consensus sequences were isolated as shown in Table 2. These results demonstrate that individual antigen-binding phage clones can be isolated from the VH library. These phage clones are highly reactive in phage ELISA assays and are specific for the antigen. DNA sequence analysis of the clones isolated after the fourth round of panning revealed that the enrichment was specific for individual clones, thus, 50-60% of the sequences obtained were identical at the expected region of CDR3.

On page 22, please replace Table 2: with the following table:

Table 2: Amino acid composition of CDR3 region of selected phage clones.

	Amino Acid									Specificity
	1	2	3	4	5	6	7	8	9	
SEQ ID NO:7	Phe	Pro	Thr	Gly	Asp	Leu	Ala	Glu	Lys	**solid TNF**
SEQ ID NO:8	Asn	**Gly**	Lys	**Ser**	**Pro**	**Gln**	Ala	Ala	Trp	**Ig**
SEQ ID NO:9	Gln	Ser	**Gly**	Gln	**Ser**	**Pro**	**Gln**	Ser	Ile	**Ig**
SEQ ID NO:10	**Trp**	**Gly**	Ser	Trp	**Arg**	Asn	**Gly**	Lys	Ans	**polystyrene**
SEQ ID NO:11	**Trp**	**Ala**	Lys	Gly	**Arg**	**Ser**	Thr	Met	Tyr	**polystyrene**
SEQ ID NO:12	**Trp**	**Gly**	Met	Tyr	**Arg**	**Ser**	**Gly**	Thr	Gly	**polystyrene**

Also on page 22, starting at line 5, please replace the paragraph starting with "For the large scale protein production" with the following new paragraph:

For large scale protein production plasmid DNA from positive binding clones was reamplified with the following oligonucleotides pET-21aVH5'NdeI (SEQ ID NO: 13) [5'-GGGAATTCCATATGGATGTCCAGCTGCAGGAGTC-3'] and pET-21aVH3'XhoI (SEQ ID NO:14) [5'GGGAATTCCTCGAGCTATGCGGCACGCGGTTCCA-3']. These inserted cloning sites that enabled subcloning into the T7 promoter-based pET-21 a expression vector (Novagen). Protein was expressed at high levels in BL21 (DE 3) cells upon IPTG induction and accumulated in intracellular inclusion bodies. Inclusion bodies were isolated and purified from the induced BL21 cells and solubilized in Guanidine HC1. Following reduction inclusion bodies were refoled in a redox-shuffling buffer system and Arginine. After refolding the protein was dialyzed and concentrated by Minisette 5K (Filtron), and purified by MonoQ (Admersham Pharmacia Biotech) ion-exchange and TSK300 gel filtration chromatography.

On page 29, please replace Table 3: with the following:

Table 3: Amino acid composition of CDR3 region of selected phage clones specific to Streptavidin.

	Clone	1	2	3	4	5	6	7	8	9
SEQ ID NO:15	**3**	His	Ala	Gln	Arg	Arg	Pro	Trp	Ile	Arg
SEQ ID NO:16	**8**	Glu	Asp	Pro	**His**	**Pro**	**Gln**	Arg	Gly	Tyr

EXHIBIT B
MARKED VERSION OF THE AMENDED CLAIMS

IN THE CLAIMS:

Please amend claims to read as follows:

7. (Amended) The polypeptide of claim 3 wherein the CDR3 sequence between residues 95 and 100C comprises the consensus sequence: Gly-X-Ser-Pro-Gln (SEQ ID NO:6), wherein X represents any amino acid.

8. (Amended) The polypeptide of claim 3 wherein the CDR3 sequence between residues 95 and 100C is selected from the sequences: Gln-Ser-Gly-Gln-Ser-Pro-Gln-Ser-Ile (SEQ ID NO:9), and Asn-Gly-Lys-Ser-Pro-Gln-Ala-Ala-Trp (SEQ ID NO:8).

10. (Amended) The polypeptide of claim 9 wherein the CDR3 sequence between residues 95 and 100C comprises the sequence: Phe-Pro-Thr-Gly-Asp-Leu-Ala-Glu-Lys (SEQ ID NO: 7).

12. (Amended) The polypeptide of claim 11 wherein the CDR3 sequence between residues 95 and 100C is selected from the sequences: His-Ala-Gln-Arg-Arg-Pro-Trp-Ile-Arg (SEQ ID NO:15), and Glu-Asp-Pro-His-Pro-Gln-Arg-Gly-Tyr (SEQ ID NO:16).